(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 684 259 A1**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **95107298.2**

(51) Int. Cl.[6]: **C07K 5/09**, A61K 38/06

(22) Date of filing: **13.05.95**

(30) Priority: **27.05.94 JP 115161/94**

(43) Date of publication of application:
29.11.95 Bulletin 95/48

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE

(71) Applicant: **HOECHST JAPAN LIMITED**
10-16, 8-chome, Akasaka, Minato-ku
Tokyo (JP)

(72) Inventor: **Koyama, Masayoshi**
1952-31 Matoba
Kawagoe-shi,
Saitama 350-11 (JP)
Inventor: **Takahashi, Mikiko**
1-11 Ayase
Hasuda-shi,
Saitama 349-01 (JP)
Inventor: **Yanagawa, Masayoshi**
2-3-1 Shinsayama
Sayama-shi,
Saitama 350-13 (JP)

(74) Representative: **Lauppe, Hans Friedrich**
Behringwerke AG
Abt. Patente & Verträge,
Postfach 1140
D-35001 Marburg (DE)

(54) L-lysyl-glycyl-L-histidine and therapeutic agent for wound healing containing the same.

(57) L-lysyl-glycyl-L-histidine and metal complexes thereof, for example, L-lysyl-glycyl-L-histidine : copper (II).
The L-lysyl-glycyl-L-histidine and salt thereof have a fibroblast proliferation promoting activity and then it is useful as a wound healing agent.

EP 0 684 259 A1

Background of the Invention

1. Field of the Invention

This invention relates to a new tripeptide having an amino acid sequence L-lysyl-glycyl-L-histidine and/or metal complexes thereof, and a pharmaceutical composition for wound healing containing the same as an active ingredient.

2. Description of the Prior Art

Various growth factors are known to be involved in the process of wound healing (Dijke et al., Biotechnology, Vol. 7, p. 793-798, 1989). TGF-β (transforming growth factor-β) and PDGF (platelet-derived growth factor), in particular, are known to proliferate fibroblasts, induce the cells to a damaged site and enhance wound repairing.

Some tripeptides and metal complexes thereof have been known to have a cell proliferation effect. For example, glycyl-L-histidyl-L-lysine, which was isolated from plasma or synthesized and metal complexes thereof, such as, glycyl-L-histidyl-L-lysine : copper (II) are involved in cell proliferation of hepatocytes, neurocytes, kidney cells, thyroid cells etc., and maintenance of growth (Picart, L. Method, Enzym. Vol. 147, p. 314-328, 1987; Picart, L. IN Vitro, Vol. 17, p. 459-466, 1981; Picart, L. US Patent No. 4,760,051). It is assumed that the cell proliferation activity of glycyl-L-histidyl-L-lysine involves binding of an essential metal ion of trace elements such as copper ion and incorporating the copper ion in cells (Picart, L. IN Vitro, Vol. 17, p. 459-466 1981). Also disclosed is a use of glycyl-L-histidyl-L-lysine and metal complexes thereof, for example, glycyl-L-histidyl-L-lysine : copper (II) as an agent for promoting a therapy of affected bones and other hard tissues such as cartilage of warm-blooded animals (Picart, L. R., Japanese Patent Publication No. Hei 5-501253).

L-lysil-L-histidyl-glycine was isolated from an avian bursa fabricii as a B cell differentiating hormone (Audhya, T. et al. Science Vol. 231, p. 997-999, 1986). Also it is disclosed that L-lysyl-L-histidyl-glycine is used as an agent for promoting a therapy for the affected bones and other hard tissues such as cartilage (Picart, L. R., the above-mentioned Patent Publication).

L-histidyl-glycyl-L-lysine and L-histidyl-L-lysyl-glycine have been isolated from the feline spinal marrow as an inhibitor of transmission of neurocytes (Lote, C.J. et al., Nature, Vol. 264, p. 188-189, 1976). On the other hand, only glycyl-L-histidyl-L-lysine is known to constitute a metal complex with a metal ion, for example, copper ion among the above-mentioned tripeptides (Picart, L., the afore-mentioned).

However, it has never been reported that L-lysyl-glycyl-L-histidine nor metal complexes thereof, for example, L-lysyl-glycyl-L-histidine : copper (II) proliferates fibroblasts or is effective for wound healing.

Detailed Description of the Invention

Consequently, an object of the invention is to provide a tripeptide having a wound healing activity.

Another object of the invention is to provide a pharmaceutical composition for wound healing containing said tripeptide as an effective ingredient.

TGF-β and PDGF, which were hitherto considered to have a wound healing effect, are physiologically active substances having various functions of themselves and are considered to have other undesirable activities than wound healing. Therefore, a low molecular peptide having no such side effects and being specifically active in wound healing has been desired for the above purposes.

The present inventors have found, as a result of various studies, the fact that L-lysyl-glycyl-L-histidine and/or metal complexes thereof such as L-lysyl-glycyl-L-histidine : copper (II) promotes fibroblast proliferation, and this invention has now been completed.

The tripeptide and metal complexes thereof according to this invention are highly water-soluble, and they are most suitably administered in combination with a suitable water-soluble base for wound healing preferably by applying locally to an affected region. Preparations for an external use according to the invention may take the form of a water-soluble ointment, an oleaginous ointment, lotion, spray, oil, gel or the like. Representative bases may include macrogols for a water-soluble ointment, vaseline for an oleaginous ointment, vegetable oils such as olive oil, sesame oil, camellia oil and the like for an oil preparation, and carboxy vinyl polymer, sodium polyacrylate and the like for a gel preparation. The tripeptide and metal complexes thereof according to the invention can also be administered intravenously or subcutaneously in systemic administration, and nasally or transpulmonarily in the form of micronized aerosols.

The dosages are in a range of 1 to 100 mg/administration site/person/day in local administration, and 0.1 to 10 mg/kg/day in systemic administration.

The following examples are provided to illustrate the invention and are not intended to limit the invention.

Examples

Example 1 Synthesis of L-lysyl-glycyl-L-histidine

A peptide consisting of L-lysyl-glycyl-L-histidine was synthesized by way of solid-phase synthesis using an automatic peptide synthesizer (Applied Biosystems, Inc., U.S.A.). Using 0.5 mM of resin comprising styrene-divinylbenzene copolymer (molar ratio; styrene : divinylbenzene = 99 : 1), amino acids were connected successively toward the N-terminus of the peptide. One mM of N-(t-butoxycarbonyl)-L-Lys,N-(t-butoxycarbonyl)-Gly and N-(t-butoxycarbonyl)-L-His, respectively, were used as amino acids in the reaction. The peptides were obtained by the detachment from the solid-phase and the removal of protecting group by using 5 ml of 95% trifluoroacetic acid (TFA). The peptides obtained were purified by HPLC (available from Hitachi Corp.), then reverse-phase C-18 column (available from Vydac, Inc.) eluting with a linear gradient of acetonitrile containing 0.1% TFA.

Example 2 Determination of fibroblast proliferation promoting activity of L-lysyl-glycyl-L-histidine

Fibroblast cell strains, Balb/3T3 cells (purchased from ATCC) were inoculated into a 96-well culture plate at 5 x 103 cells/well, 100 µl of 10% calf serum-containing Dulbecco Modified Eagle Medium (hereinafter referred to as DME) was added and incubated at 370C for 24 hours in an incubator. Then, the culture medium was removed and the cells were washed. One hundred µl of lowered serum medium (0.2% calf serum-containing DME) was added to the wells and incubation was continued for another 3 days. L-lysyl-glycyl-L-histidine obtained in Example 1 was added thereto at 10 µl/well and incubation was performed for 15 hours.. The culture medium contains a trace amount of Cu2+, and a part of L-lysyl-glycyl-L-histidine may form copper complexes. The 3H-thymidine was added to be 74 KBq/ml and incubation was performed for 6 hours. After completion of the incubation, the medium was removed, the cells were collected and the amount of 3H-thymidine incorporated in the cells was determined.

The results of the determination of fibroblast proliferation promoting activity of L-lysyl-glycyl-L-histidine are shown in Table 1, wherein the data show the mean and its standard deviation (4 cases per one group).

Table 1

Added Compound	Dose (M)	Incorporated 3H-thymidine (cpm)
Control	-	1392.3 ± 348.2
L-lysyl-glycyl-L-histidine	10^{-6}	1406.5 ± 470.5
	10^{-5}	1371.8 ± 249.1
	10^{-4}	4889.8 ± 495.1
	10^{-3}	7719.5 ± 1246.8

From the results, it was confirmed that L-lysyl-glycyl-L-histidine has a dose-dependent cell proliferation promoting activity.

Claims

1. A tripeptide comprising an amino acid sequence L-lysyl-glycyl-L-histidine and/or a metal complex thereof.

2. The tripeptide according to claim 1 comprising an amino acid sequence L-lysyl-glycyl-L-histidine.

3. The tripeptide metal complex according to claim 1 comprising L-lysyl-glycyl-L-histidine : copper (II).

4. A pharmaceutical composition for wound healing containing an effective amount of a tripeptide comprising an amino acid sequence L-lysyl-glycyl-L-histidine and/or a metal complex thereof as an active ingredient.

5. The pharmaceutical composition for wound healing according to claim 4 wherein the tripeptide comprises an amino acid sequence L-lysyl-glycyl-L-histidine.

6. The pharmaceutical composition for wound healing according to claim 4 wherein the tripeptide metal complex comprises L-lysyl-glycyl-L-histidine : copper (II).

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 95 10 7298

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	WO-A-91 03488 (PROCYTE CORP) 21 March 1991 * table 3 * ---	1,3,4,6	C07K5/09 A61K38/06
A	WO-A-88 08851 (PROCYTE CORP) 17 November 1988 * the whole document * ---	1-6	
A	J.CELL.BIOL., vol. 125, no. 4, May 1994 pages 929-943, T.F.LANE C.S. 'SPARC is a source of copper-binding peptides that stimulate angiogenesis' * the whole document * -----	1-6	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C07K
A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	6 September 1995	Groenendijk, M

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C08)